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(54) Title: POROUS DELIVERY SCAFFOLD AND METHOD
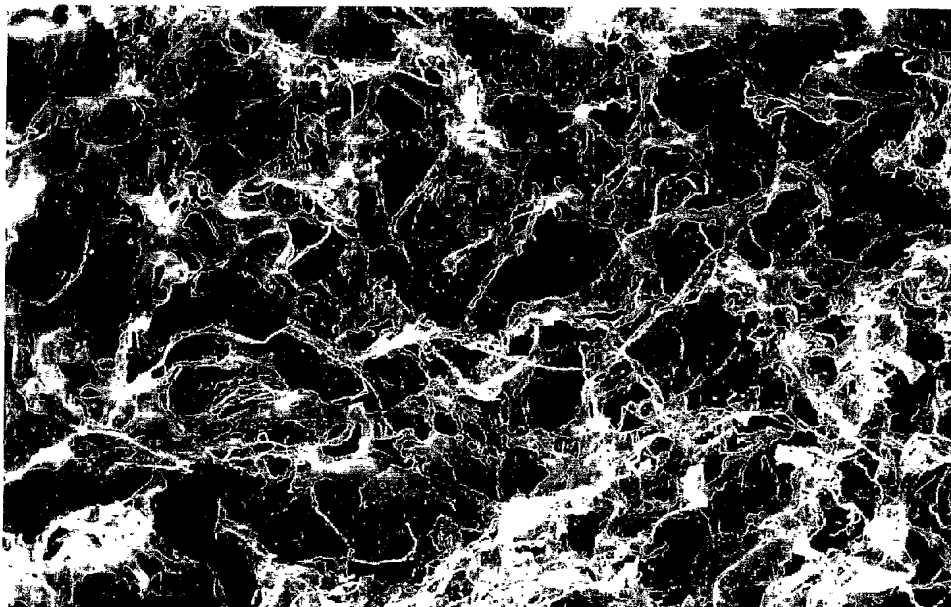
(57) **Abstract:** A method of making an implantable scaffold for repairing damaged or diseased tissue includes the step of suspending pieces of an extracellular matrix material in a liquid. The extracellular matrix material and the liquid are formed into a mass. The liquid is subsequently driven off so as to form interstices in the mass. The scaffold may further comprise biological agents that promote tissue repair and healing. Porous implantable scaffolds fabricated by such a method are also disclosed.

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POROUS DELIVERY SCAFFOLD AND METHOD

CROSS REFERENCE TO RELATED APPLICATIONS

5   Cross reference is made to copending U.S. patent applications Serial
No. XX/XXX,XXX entitled "Meniscus Regeneration Device and Method" (Attorney
Docket No. 265280-71141, DEP-745); Serial No. XX/XXX,XXX entitled "Devices
from Naturally Occurring Biologically Derived Materials" (Attorney Docket No.
265280-71142, DEP-748); Serial No. XX/XXX,XXX entitled "Cartilage Repair
Apparatus and Method" (Attorney Docket No. 265280-71143, DEP-749); Serial No.
10   XX/XXX,XXX entitled "Unitary Surgical Device and Method" (Attorney Docket No.
DEP-750); Serial No. XX/XXX,XXX entitled "Hybrid Biologic/Synthetic Porous
Extracellular Matrix Scaffolds" (Attorney Docket No. 265280-71144, DEP-751);
Serial No. XX/XXX,XXX entitled "Cartilage Repair and Regeneration Device and
Method" (Attorney Docket No. 265280-71145, DEP-752); Serial No. XX/XXX,XXX
15   entitled "Porous Extracellular Matrix Scaffold and Method" (Attorney Docket No.
265280-71146, DEP-747); and Serial No. XX/XXX,XXX entitled "Cartilage Repair
and Regeneration Scaffolds and Method" (Attorney Docket No. 265280-71180, DEP-
763), each of which is assigned to the same assignee as the present application, each
of which is filed concurrently herewith, and each of which is hereby incorporated by
20   reference. Cross reference is also made to U.S. Patent Application Serial No.
10/172,347 entitled "Hybrid Biologic-Synthetic Bioabsorbable Scaffolds" which was
filed on June 14, 2002, which is assigned to the same assignee as the present
application, and which is hereby incorporated by reference.

25   FIELD OF THE DISCLOSURE
        The present disclosure relates generally to an extracellular matrix
scaffold, and more particularly to a porous extracellular matrix scaffold for repairing
or regenerating body tissue and a method for making such a scaffold.

30   BACKGROUND OF THE INVENTION
        Naturally occurring extracellular matrices (ECMs) are used for tissue
repair and regeneration. One such extracellular matrix is small intestine submucosa
(SIS). SIS has been used to repair, support, and stabilize a wide variety of anatomical

defects and traumatic injuries. Commercially-available SIS material is derived from porcine small intestinal submucosa that remodels to the qualities of its host when implanted in human soft tissues. Further, it is taught that the SIS material provides a natural matrix with a three-dimensional microstructure and biochemical composition

5   that facilitates host cell proliferation and supports tissue remodeling. Indeed, SIS has been shown to contain biological molecules, such as growth factors and glycosaminoglycans that aid in the repair of soft tissue of the human body. The SIS material currently being used in the orthopaedic field is provided in a dried and layered configuration in the form of a patch to repair or regenerate soft tissue such as

10   tendons, ligaments and rotator cuffs.

       While small intestine submucosa is readily available, other sources of submucosa are known to be effective for tissue remodeling. These sources include, but are not limited to, stomach, bladder, alimentary, respiratory, or genital submucosa, or liver basement membrane. See, e.g., U.S. Patent Nos. 6,379,710, 6,171,344;

15   6,099,567; and 5,554,389, each of which is hereby incorporated by reference.

       Further, while SIS is most often porcine derived, it is known that various submucosa materials may also be derived from non-porcine sources, including bovine and ovine sources. Additionally, the ECM material may also include partial layers of laminar muscularis mucosa, muscularis mucosa, lamina

20   propria, stratum compactum and/or other such tissue materials depending upon factors such as the source from which the ECM material was derived and the delamination procedure.

       As used herein, it is within the definition of a naturally occurring extracellular matrix to clean, delaminate, and/or comminute the extracellular matrix,

25   or to cross-link the collagen or other components within the extracellular matrix. It is also within the definition of naturally occurring extracellular matrix to fully or partially remove one or more components or subcomponents of the naturally occurring matrix. However, it is not within the definition of a naturally occurring extracellular matrix to separate and purify the natural components or subcomponents

30   and reform a matrix material from purified natural components or subcomponents. Thus, while reference is made to SIS, it is understood that other naturally occurring extracellular matrices (e.g., stomach, bladder, alimentary, respiratory, and genital submucosa, and liver basement membrane), whatever the source (e.g., bovine,

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porcine, ovine) are within the scope of this disclosure. Thus, in this application, the terms "naturally occurring extracellular matrix" or "naturally occurring ECM" are intended to refer to extracellular matrix material that has been cleaned, processed, sterilized, and optionally cross-linked. The following patents, hereby incorporated by reference, disclose the use of ECMs for the regeneration and repair of various tissues: 6,379,710; 6,187,039; 6,176,880; 6,126,686; 6,099,567; 6,096,347; 5,997,575; 5,993,844; 5,968,096; 5,955,110; 5,922,028; 5,885,619; 5,788,625; 5,762,966; 5,755,791; 5,753,267; 5,733,337; 5,711,969; 5,645,860; 5,641,518; 5,554,389; 5,516,533; 5,460,962; 5,445,833; 5,372,821; 5,352,463; 5,281,422; and 5,275,826.

The manipulation of scaffold pore size, porosity, and interconnectivity is an important science contributing to the field of tissue engineering (Ma and Zhang, 2001, J Biomed Mater Res, 56(4):469-477; Ma and Choi, 2001 Tissue Eng, 7(1):23-33) because it is believed that the consideration of scaffold pore size and density/porosity influences the behavior of cells and the quality of tissue regenerated. In fact, several researchers have shown that different pore sizes influence the behavior of cells in porous three-dimensional matrices. For example, it has been demonstrated in the art that for adequate bone regeneration to occur scaffold pore size needs to be at least 100 microns (Klawitter et al., 1976, J Biomed Mater Res, 10(2):311-323). For pore sizes and interconnectivity less than that, poor quality bone is regenerated and if pore size is between 10-40 microns bone cells are able to form only soft fibro-vascular tissue (White and Shors, 1991, Dent Clin North Am, 30:49-67). The consensus of research for bone regeneration indicates that the requisite pore size for bone regeneration is 100 – 600 microns (Shors, 1999, Orthop Clin North Am, 30(4):599-613; Wang, 1990, Nippon Seikeigeka Gakki Zasshi, 64(9):847-859). It is generally known in the art that optimal bone regeneration occurs for pore sizes between 300 – 600 microns.

Similarly, for the regeneration of soft orthopaedic tissues, such as ligament, tendon, cartilage, and fibro-cartilage, scaffold pore size is believed to have a substantial effect. For example, basic research has shown that cartilage cells (chondrocytes) exhibit appropriate protein expression (type II collagen) in scaffolds with pore sizes of the order of 20 microns and tend to dedifferentiate to produce type I collagen in scaffolds with nominal porosity of about 80 microns (Nehrer et al., 1997, Biomaterials, 18(11):769-776). More recently, it has been shown that cells that form

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ligaments, tendons, and blood vessels (fibroblasts and endothelial cells) exhibit significantly different activity when cultured on scaffolds with differing pore sizes ranging from 5 to 90 microns (Salem et al., 2002, J Biomed Mater Res, 61(2):212-217).

5

SUMMARY

According to one illustrative embodiment, there is provided a method of making an implantable scaffold for repairing damaged or diseased tissue. The method includes the step of suspending, mixing, or otherwise placing pieces of a
10 naturally occurring extracellular matrix material in a liquid. The naturally occurring extracellular matrix material and the liquid are formed into a mass. The liquid is subsequently driven off so as to form interstices in the mass. In one specific implementation of this exemplary embodiment, the liquid is driven off by freeze drying the naturally occurring extracellular matrix material and the liquid in which it
15 is suspended. In such a manner, the liquid is sublimed thereby forming the interstices in the mass.

The material density and pore size of the scaffold may be varied by controlling the rate of freezing of the suspension. The amount of water into which the pieces of naturally occurring extracellular matrix material is suspended may also be
20 varied to control the material density and pore size of the resultant scaffold.

In a further illustrative embodiment, biological agents that promote tissue repair and/or healing are incorporated into the scaffold. The scaffold is either cross-linked chemically or enzymatically. The biological agents may be present during cross-linking of the scaffold, thereby becoming physically entrapped in the
25 cross-linked scaffold. Furthermore, the biological agent may be protected by liposomar delivery. Alternatively, the biological agents may be perfused into the scaffold after cross-linking has occurred. Controlled release of the biological agents from the scaffold is then achieved by diffusion.

The biological agent may also be covalently attached to the scaffold.
30 The biological agent is attached either chemically or enzymatically. The biological agent may be attached without further modification or it may be conjugated with a spacer arm. Controlled release of the biological agents occurs when the scaffold is resorbed or otherwise biologically degraded. Alternatively, if a spacer arm is used,

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the spacer arm may have a site that allows for cleavage of the spacer arm under discreet biological conditions. Upon cleavage of the spacer arm, the biological agents would then be free to diffuse from the scaffold.

5   In accordance with another exemplary embodiment, there is provided an implantable scaffold for repairing or regenerating tissue which is prepared by the process described above.

In another aspect, the present disclosure provides an implantable scaffold for repairing or regenerating body tissue. The scaffold comprises a porous body of naturally occurring extracellular matrix pieces that are interconnected to

10   define an interior surface of the body. The interior surface has a three-dimensional topography of irregular shape.

In another aspect, the present disclosure provides an implantable device for repairing or regenerating body tissue. The device comprises a three-dimensional reticulated foam comprising a plurality of interconnected pores. The

15   interconnected pores define three-dimensional interconnected passageways having irregular shapes. At least part of the reticulated foam comprises naturally occurring extracellular matrix.

The devices described above may further include biological agents that aid in tissue repair or healing. The devices provide controlled release of the

20   biological agents at the implantation site.

In another aspect, the present disclosure provides a method of making an implantable device for repairing or regenerating body tissue. The method comprises the steps of providing a naturally occurring extracellular matrix material in a raw form, comminuting the raw naturally occurring extracellular matrix in the

25   presence of a liquid to form a slurry of naturally occurring extracellular matrix, and lyophilizing the slurry of naturally occurring extracellular matrix to form a reticulated foam of naturally occurring extracellular matrix.

In another aspect, the present disclosure provides a method of making an implantable scaffold for repairing or regenerating body tissue. The method

30   comprises the steps of providing a naturally occurring extracellular matrix material in a raw form, comminuting the raw naturally occurring extracellular matrix to form cohesive pieces of naturally occurring extracellular matrix, and lyophilizing the

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cohesive pieces of naturally occurring extracellular matrix to form a reticulated foam of naturally occurring extracellular matrix.

The above methods may further comprise the step of cross-linking the reticulated foam in the presence of a biological agent, thereby trapping the biological agent in the scaffold. Alternatively, the reticulated foam is cross-linked and the biological agent is subsequently perfused into the cross-linked foam. The scaffold may be cross-linked either chemically or enzymatically.

Alternatively, the above methods may further comprise the step of covalently attaching a biological agent to the reticulated foam. The biological agent may be covalently attached either chemically or enzymatically. Furthermore, the biological agent may be conjugated with a spacer arm.

The implantable devices disclosed herein are three dimensional, porous scaffolds of ECMs like SIS. As such, it is evident that an implant based on the teachings of the present disclosure will have the dual advantage of having not only the appropriate biochemistry (collagens, growth factors, glycosaminoglycans, etc. naturally found in such ECMs and/or any exogenously added biologically agents that are coupled with or trapped in the implant) but also the appropriate physical microstructure to enable desired cellular activity upon implantation. These implantable devices are likely to find therapeutic use in the orthopaedic field, for devices used in the treatment of diseased or damaged fibro-cartilage such as the meniscus, diseased or damaged articular cartilage, diseased or damaged bone, diseased or damaged ligaments, tendons, or other connective tissues in the body.

The above and other features of the present disclosure will become apparent from the following description and the attached drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

The detailed description particularly refers to the accompanying figures in which:

FIG. 1 is an image from a scanning electron microscope which shows the surface of a porous reticulated SIS open cell foam scaffold having a relatively large pore size and a relatively low material density;

FIG. 2 is an image from a scanning electron microscope which shows the surface of a porous reticulated SIS open cell foam scaffold having a relatively moderate pore size and a relatively moderate material density;

FIG. 3 is an image from a scanning electron microscope which shows the surface of a porous reticulated SIS open cell foam scaffold having a relatively small pore size and a relatively high material density;

FIG. 4 is an image from a scanning electron microscope which shows a cross-section of a porous reticulated SIS open cell foam scaffold, with an example of a pore indicated by the arrow, the image being at a greater magnification than the images of FIGS. 1-3;

FIG. 5 is an image from a scanning electron microscope which shows a cross-section of a porous reticulated SIS open cell foam scaffold, with examples of pores indicated by the arrows, the image being at a greater magnification than the images of FIGS. 1-3;

FIG. 6 is an image from a scanning electron microscope which shows a cross-section of a porous reticulated SIS open cell foam scaffold, with examples of pores indicated by the arrows, the image being at a greater magnification than the images of FIGS. 1-3;

FIG. 7 is an image from a scanning electron microscope which shows a cross-section of a porous reticulated SIS open cell foam scaffold, the image being at a greater magnification than the images of FIGS. 1-3;

FIG. 8 is an image from a scanning electron microscope which shows a surface of a porous reticulated SIS open cell foam scaffold, the image being at a greater magnification than the images of FIGS. 1-3;

FIGS. 9 and 10 are images from a scanning electron microscope which show a mass of cohesive SIS pieces; and

FIG. 11 is a bar graph which shows the controlled diffusion of covalently linked FITC-BSA from a porous reticulated SIS open cell foam scaffold that has been cross-linked in the presence of FITC-BSA.

DETAILED DESCRIPTION OF THE DRAWINGS

The present disclosure relates to a porous scaffold for implanting into the body of a patient to repair or regenerate damaged or diseased tissue. The porous

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scaffold is constructed from a naturally occurring extracellular material. For example, the scaffold may be constructed from SIS. As will be discussed herein in greater detail, both the material density and the pore size of the porous scaffold may be varied to fit the needs of a given scaffold design.

5   Such porous scaffolds may be fabricated by suspending pieces of an extracellular matrix material in a liquid. As used herein, the term "piece" is intended to mean any fiber, strip, ribbon, sliver, filament, shred, bit, fragment, part, flake, slice, cut, chunk, or other portion of solid or solid-like material. Also, as used herein, the term "suspending" is intended to include any placement of a solid (e.g., pieces of

10   ECM) in a liquid whether or not an actual suspension is created. As such, the term "suspending" is intended to include any mixing of a solid in a liquid or any other placement of a solid in a liquid. As a result, the term "suspension" is likewise not intended to be limited to suspensions, but rather is intended to mean any mass having a solid present in a liquid.

15   In any event, the suspension of the pieces of extracellular matrix material and the liquid forms a mass in the form of, for example, a "slurry". The liquid may then be subsequently driven off of the mass so as to form interstices therein. The liquid may be driven off in a number of different manners. For example, as will herein be described in greater detail, the liquid may be driven off via

20   sublimation in a freeze drying process. Alternatively, the liquid may also be driven off by subjecting the suspension to either an unheated vacuum process or a vacuum under a controlled heating process. The liquid may also be driven off from the suspension ultrasonically. Microwave energy, RF energy, UV energy, or any other type of energy (or combination thereof) may also be utilized to drive the liquid off of

25   the suspension. Liquid may also be driven off of the suspension by forcing or drawing air through the suspension. The suspension may be centrifuged to drive off the liquid. Moreover, the liquid may include a water-soluble filler which is driven off, for example, by use of an alcohol. In short, the present disclosure contemplates the driving off of the liquid from the suspension by any liquid removal process.

30   As alluded to above, while any of the aforementioned processes for driving off the liquid from the suspension may be utilized, along with any other process known by one skilled in the art, the processes of the present disclosure will herein be exemplary described in regard to a lyophilization process (i.e., freeze

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drying). However, it should be understood that such a description is merely exemplary in nature and that any one or more of the aforedescribed processes for driving off the liquid from the suspension may be utilized to fit the needs of a given scaffold design or process design.

5   As alluded to above, one useful process for fabricating the porous scaffolds of the present disclosure is by lyophilization. In this case, pieces of an extracellular matrix material are suspended in a liquid. The suspension is then frozen and subsequently lyophilized. Freezing the suspension causes the liquid to be turned to ice crystals. These ice crystals are then sublimed under vacuum during the

10   lyophilization process thereby leaving interstices in the material in the spaces previously occupied by the ice crystals. The material density and pore size of the resultant scaffold may be varied by controlling, amongst other things, the rate of freezing of the suspension and/or the amount of water in which the extracellular matrix material is suspended in at the on-set of the freezing process.

15   As a specific example of this process, fabrication of a porous SIS scaffold by lyophilization will be described in detail. However, it should be appreciated that although the example is herein described in regard to an SIS scaffold, fabrication of a scaffold constructed from other extracellular matrix materials may also be performed in a similar manner.

20   The first step in fabricating a porous scaffold with a desired pore size and density is the procurement of comminuted SIS. Illustratively, scissor-cut SIS runners (~6" long) are positioned in a 1700 series Comitrol™ machine, commercially available from Urschel Laboratories (Valparaiso, Indiana). The SIS material is processed in the presence of a liquid and thereafter collected in a receptacle at the

25   output of the machine. The material is then processed through the machine a second time under similar conditions. In one exemplary process, a liquid (e.g., water) is introduced into the input of the machine contemporaneously with the SIS material. The resultant material is a "slurry" of SIS material (thin, long SIS fibers ~200 microns thick x 1-5 mm long) suspended in a substantially uniform manner in water.

30   Although the suspension is herein described as being formed as a byproduct of the comminuting process, it should be appreciated that the pieces of SIS may be suspended in the liquid (i.e., water) in other manners known to those skilled in the art. Furthermore, while other methods are known for comminuting SIS, it is understood

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that for the purposes of the present disclosure, comminuted SIS comprises, ribbon-like or string-like fibers wherein at least some of the individual pieces of ECM and SIS material have lengths greater than their widths and thicknesses. Such fibers may be interlaced to provide a felt-like material, if desired.

5   Process parameters can be varied using the above-identified 1700 series Comitrol™ machine, including the choice of blade used, whether water is used, the amount of water used, the speed at which the blades turn, and the number of times the material is passed through the machine. As an example, cutting head 140084-10 and a Vericut, sealed impeller from Urschel Laboratories may be used, with a flow of

10   water of about two (2) gallons per minute, with the blade running at a constant speed of about 9300 rpm. A first pass through the machine at these parameters will produce fibrous SIS material of varying sizes, and a second pass will produce SIS fibers of a more uniform size. By way of example, the comminuted material may be tested to determine if it has the consistency of that which is desired for use in regard to the

15   illustrative embodiments described herein by the following process: the comminuted SIS suspension or slurry is centrifuged, excess water is poured off and the remaining slurry is poured into a dish. By hand, a small amount of the comminuted SIS material in the dish is pinched between the thumb and index finger and gently lifted from the dish. Illustratively, at least a small amount of additional SIS, beyond the portion

20   pinched between the thumb and index finger, will lift along with the material that has been pinched ("pinch test"). This additional comminuted SIS material lifts with the material that is between the thumb and index finger because the individual pieces of comminuted SIS material are commingled or intertwined.

          The terms "cohesive ECM", "cohesive SIS", "cohesive ECM pieces"

25   and "cohesive SIS pieces" are used herein to respectively denote ECM or SIS material that has been comminuted or otherwise physically processed to produce ECM or SIS pieces that are capable of comingling or intertwining (in the wet or dry state) to form a mass of discrete pieces of ECM or SIS that remain massed together under some conditions (such as under gravity), regardless of the shape or shapes of the individual

30   ECM or SIS pieces. One method of demonstrating that the ECM or SIS material comprises cohesive pieces is the "pinch test" described in the preceding paragraph. Examination of the final ECM or SIS product produced may also provide evidence that the base material comprised cohesive ECM or SIS pieces. Illustratively, the

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ECM or SIS pieces are sufficiently cohesive to each other (or to other pieces in the mix or slurry) that they remain unified throughout the process used to produce the foam structure. Examples of cohesive SIS pieces are shown in the scanning electron microscopic images of FIGS. 9 and 10.

5   Thereafter, the comminuted SIS suspension is frozen and lyophilized (i.e., freeze dried). In particular, the SIS suspension is frozen at a controlled rate of temperature drop to control the size of the formed ice crystals. Once frozen, and without allowing the material to thaw, the lyophilization process sublimes the ice crystals directly to a vapor under vacuum and low temperatures. This leaves voids or

10   interstices in the spaces previously occupied by the ice crystals.

Any commercially available freezer for freezing the suspension to a desired temperature may be used. Likewise, any commercially available lyophilizer may be used for the lyophilization process. One exemplary machine for performing the lyophilization process is a Virtis Genesis™ Series lyophilizer which is

15   commercially available from SP Industries, Inc. of Gardiner, New York.

The process parameters of the aforedescribed fabrication process may be varied to produce scaffolds of varying pore sizes and material densities. For example, the rate at which the suspension is frozen, the amount of water present in the suspension, or the compactness of the extracellular matrix material may be varied to

20   produce scaffolds of varying pore sizes and material densities.

For instance, to produce scaffolds having a relatively large pore size and a relatively low material density, the extracellular matrix suspension may be frozen at a slow, controlled rate (e.g., $-1°C/min$ or less) to a temperature of about $-20°C$, followed by lyophilization of the resultant mass. To produce scaffolds having a

25   relatively small pore size and a relatively high material density, the extracellular matrix material may be tightly compacted by centrifuging the material to remove a portion of the liquid (e.g., water) in a substantially uniform manner prior to freezing. Thereafter, the resultant mass of extracellular matrix material is flash-frozen using liquid nitrogen followed by lyophilization of the mass. To produce scaffolds having a

30   moderate pore size and a moderate material density, the extracellular matrix material is first tightly compacted by centrifuging the material to remove a portion of the liquid (e.g., water) in a substantially uniform manner prior to freezing. Thereafter, the resultant mass of extracellular matrix material is frozen at a relatively fast rate (e.g., >

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−1°C/min) to a temperature of about −80°C followed by lyophilization of the mass.

EXAMPLE 1

Example 1 demonstrates the fabrication of a porous SIS scaffold

5   having a relatively large pore size and a relatively low material density. Such

scaffolds are obtained by freezing a comminuted SIS suspension at a slow, controlled

rate (−1°C/min or less) to −20°C, followed by lyophilization. The procedure is as

follows. First, comminuted SIS is fabricated as described above. Specifically,

scissor-cut SIS runners (~6" long) are placed in a suitable comminuting machine such

10   as the Urschel Comitrol machine described above. The comminuted SIS is collected

in a receptacle at the output of the machine. The collected material is then processed

through the machine a second time, under the same conditions as before. The

resultant mass is a "slurry" of SIS material (thin, long SIS fibers ~200 microns thick x

1-5 mm long) suspended relatively uniformly in water.

15   Next, a slow-freeze ethanol bath is prepared as follows. Pour enough

ethanol to obtain about a 1 centimeter head in a flat-bottomed plastic container large

enough to hold four 24-well culture plates. Place the container in a -20°C freezer.

The mass of each of four empty twenty-four well plates is then recorded. Under a

sterile hood using sterile conditions, an approximately 3ml aliquot of the comminuted

20   SIS material is placed in each well of the tissue culture plates. The mass of each full

plate of material is then recorded. The four culture plates are then placed into the

ethanol freeze bath and allowed to freeze overnight.

The frozen plates are then removed from the ethanol bath and placed in

a suitable lyophilization machine such as the Virtis Genesis Series lyophilizer

25   described above. Without allowing the frozen SIS material to thaw, the process of

lyophilization sublimes ice crystals directly to vapor under vacuum and low

temperatures. This leaves voids or interstices in the spaces previously occupied by

the ice crystals. In this case, the parameters used in the lyophilization process include

a first period at a primary drying temperature of 13°C for 8 hours, followed by a

30   second period at a secondary drying temperature of 35°C for 4 hours.

After the lyophilization cycle is complete, the plates are removed from

the lyophilization machine and the mass of each plate is determined and recorded.

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The results from this process are summarized in the following table:

Average Volume	Average Mass	Average Density
1.249 ml	0.007396 g	0.006 (g/cc)

A scanning electron image of the surface of the samples was taken to visualize the relative pore sizes. These pore sizes are about 600 microns to about 700 microns. An image indicative of the samples prepared in accordance with Example 1 is shown in FIG. 1.

Pore sizes can be determined from scanning electron microscope images of the exterior surface of the foam, as in FIGS. 1-3 and 8, and of cross-sections of the foam, as in FIGS. 4-7. These images may be used in conjunction with standard commercially available image analysis software to determine the ranges of pore sizes. FIGS. 4-6 illustrate the results of using suitable commercially-available software to measure or estimate the pore sizes in the foam. This technique was used to determine that the Example 1 foam had pores in the range of 600-700 microns. The sample may also include smaller pores.

EXAMPLE 2

Example 2 demonstrates the fabrication of a porous SIS scaffold having a relatively moderate pore size and a relatively moderate material density. Such scaffolds are obtained by compacting the comminuted SIS material by centrifugation, freezing at a faster rate (relative to Example 1) and to a lower temperature (i.e., to –80°C), followed by lyophilization of the resultant mass. The procedure is as follows. First, comminuted SIS is fabricated as described above in regard to Example 1. Specifically, scissor-cut SIS runners (~6" long) is comminuted by two passes through a suitable comminuting machine to produce a "slurry" of SIS material (thin, long SIS fibers ~200 microns thick x 1-5 mm long) suspended relatively uniformly in water.

Next, the mass of each of four empty twenty-four well plates is recorded. Under a sterile hood using sterile conditions, an approximately 3ml aliquot of the comminuted SIS material is placed in each well of the tissue culture plates. The mass of each plate full of material is then recorded.

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The plates are then balanced for centrifuging by use of the following technique. The two plates are placed on the balance, and RO water is added to the area in between the wells of the lighter plate until the two plates are balanced. The two plates are then placed across from one another in the centrifuge, and centrifuged at 3000rpm for seven minutes. Once done, the plates are removed from the centrifuge, and the water is emptied therefrom. The mass of each of the plates is then recorded. The centrifuging and mass measurement process is then repeated for the remaining plates.

The plates are then placed in a -80°C freezer until the specimen is fully frozen. Depending upon the bulk of the material, the time for full freezing can vary from about 1 to about 30 minutes, for example. The frozen plates are then removed from the freezer and placed in a suitable lyophilization machine and lyophilized under similar parameters to as described above in regard to Example 1 (i.e., for a first period at a primary drying temperature of 13°C for 8 hours, followed by a second period at a secondary drying temperature of 35°C for 4 hours).

After the lyophilization cycle is complete, the plates are removed from the lyophilization machine and the mass of each plate is determined and recorded. The results from this process are summarized in the following table:

Average Volume	Average Mass	Average Density
0.285 ml	0.010104 g	0.035 g/cc

As with the samples of Example 1, a scanning electron image of the surface of each of the samples prepared in accordance with Example 2 was taken to visualize the relative pore sizes. An image indicative of the samples prepared in accordance with Example 2 is shown in FIG. 2. Using the technique described above for determining pore size, this sample was found to have pores in the range of about 100-150 microns.

EXAMPLE 3

Example 3 demonstrates the fabrication of a porous SIS scaffold having a relatively small pore size and a relatively high material density. Such scaffolds are obtained by compacting the comminuted SIS material to an even higher density than in Example

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2, flash-freezing the samples using liquid nitrogen, followed by lyophilization. The procedure is as follows. First, comminuted SIS is fabricated as described above in regard to Examples 1 and 2. Specifically, scissor-cut SIS runners (~6" long) is comminuted by two passes through a suitable comminuting machine to produce a

5   "slurry" of SIS material (thin, long SIS fibers ~200 microns thick x 1-5 mm long) suspended relatively uniformly in water. Once done, the resultant mass is centrifuged under a dead-weight. The dead weights are prepared as follows. Forty-eight strips of Coban are cut into pieces that measure 50mm in length and 5mm in width (unstretched). Thereafter, the pieces are stretched and wrapped around the outer

10   edges of a polyethylene disk measuring 1cm in diameter and 2 mm in thickness. Each strip is trimmed, if need be, so that the Coban strips wrap around the disk two times. Next, the mass of each of four empty twenty-four well plates is recorded. Under a sterile hood using sterile conditions, an approximately 3ml aliquot of the comminuted SIS material is placed in each well of the tissue culture plates. The mass of each full

15   plate of material is then recorded. The plates are then balanced and centrifuged as described above in regard to Example 2. Thereafter, the water is drained from the plates, and the mass of each of the centrifuged plates is recorded.

Once this is completed, the wells of the plates are combined at a ratio of 2:1 thereby reducing the number of plates from four to two. An attempt is made to combine low

20   material wells with high material wells in order to have a somewhat consistent amount of SIS material in each well. The mass of each full plate is then recorded. The Coban-wrapped polyethylene disks are then placed into each well. The two plates are then balanced using the technique described above in regard to Example 2. The plates are then centrifuged at 3000rpm for five minutes. Thereafter, the plates are

25   removed from the centrifuge, the water is emptied therefrom, and the polyethylene disks are also removed. The mass of each plate is again recorded. The two plates are balanced once again (in a manner similar to as described above in regard to Example 2), and the plates are again centrifuged at 3000rpm for seven minutes. Once done, the water is emptied again from each of the plates, and the mass of each plate is again

30   recorded.

Contemporaneously with the centrifuging process, a liquid nitrogen bath is prepared by pouring liquid nitrogen into a wide-mouthed liquid nitrogen container. The plates are kept in the centrifuge until the bath is ready. Thereafter,

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each plate is dipped into the bath and held in the liquid for approximately 15 seconds. Upon removal from the nitrogen bath, the plates are immediately placed in a -80°C freezer to prevent thawing. The frozen plates are then removed from the freezer and placed in a suitable lyophilization machine and lyophilized under similar parameters
5   to as described above in regard to Examples 1 and 2 (i.e., for a first period at a primary drying temperature of 13°C for 8 hours, followed by a second period at a secondary drying temperature of 35°C for 4 hours).

After the lyophilization cycle is complete, the plates are removed from the lyophilization machine and the mass of each plate is determined and recorded.
10   The results from this process are summarized in the following table:

Average Volume	Average Mass	Average Density
0.786 ml	0.071563 g	0.091 g/cc

As with the samples of Examples 1 and 2, a scanning electron image of the surface of each of the samples prepared in accordance with Example 3 was taken
15   to visualize the relative pore sizes. An image indicative of the samples prepared in accordance with Example 3 is shown in FIG. 3. Using the technique described above for determining pore size, this sample was found to have pores in the range of about 40-60 microns. Such pore sizes are illustrated in FIGS. 4-6, indicated by the arrows.

As shown in the scanning electron microscope images of FIGS. 1-8,
20   each of the illustrated ECM foams comprises a three-dimensional network of reticulated naturally occurring ECM defining a plurality of interconnected pores. The foam has these pores throughout its height, width, and thickness. The pores are open and interconnected to define a plurality of irregularly-shaped interconnected passageways leading from the exterior surface of the foam (see FIGS. 1-3 and 8) into
25   the interior of the foam (see cross-sections FIGS. 4-7). These interconnected passageways are three-dimensional. As discussed above, the sizes of the pores, and therefore the maximum size for the interconnected passageways, can be controlled by controlling the manufacturing process as described above.

These interconnected passageways facilitate cell migration through the
30   implant and enable efficient nutrient exchange *in vivo*. These interconnected passageways also provide a means of transmitting bioactive agents, biologically

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derived substances (e.g., stimulants), cells and/or biological lubricants, biocompatible inorganic materials, synthetic polymers and biopolymers (e.g., collagen) throughout the length, width and thickness of the ECM prior to implantation. The interconnected passageways defined by the pores also serve as passageways for materials used during

5   the manufacturing process, such as compounds used for chemically cross-linking the foam. These interconnected passageways as well as the outer surfaces of the foam may also serve as sites on which the above materials are carried.

As shown in the above examples, the process parameters can be varied to produce an ECM foam that has the desired porosity for the particular application.

10   For example, it may be desirable to produce a foam with lower density (and higher porosity) for applications involving osteocytes and to produce a foam with higher density (and lower porosity) for applications involving chondrocytes.

Moreover, the ECM foams described herein may be cross-linked. Specifically, the ECM foams described herein may be either chemically or physically

15   cross-linked.

As can be seen in the scanning electron microscopic images of FIGS. 1-8, each of the illustrated ECM foams comprises interconnected pieces of naturally occurring extracellular matrix. As shown in the scanning electron microscope images of cross-sections of the ECM foams of FIGS. 4-7, these interconnected pieces of

20   naturally occurring extracellular matrix provide the foam with an interior surface having an three-dimensional topography of irregular shape. As shown in the scanning electron microscope images of the surfaces of the ECM foam, these interconnected pieces of naturally occurring extracellular matrix provide the foam with exterior surfaces having three-dimensional topographies of irregular shapes. With these

25   irregular three-dimensional topographies and the interconnected passageways, the ECM foams of the present disclosure provide a relatively large surface area of naturally occurring ECM. Such a large surface area of naturally occurring ECM can be advantageous in providing a relatively large surface area to which biological agents, biologically derived agents, cells, biocompatible polymers and biocompatible

30   inorganic materials can be affixed pre-implantation. In addition, the illustrated ECM foams provide a relatively large surface of area of naturally occurring ECM to which cells may attach *in vivo*.

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ECM foam products can be made with substantially lower densities than those of other ECM products. For comparison, the density of the commercially available RESTORE® product, an ECM laminate, is 0.466 +/- 0.074 g/cc. An ECM product consisting of comminuted and hardened SIS as described in U.S. Patent

5   Application Serial No. XX/XXX,XXX entitled "Devices from Naturally Occurring Biologically Derived Materials" (Attorney Docket No. 265280-71142, DEP-748), has been made with a density of can be 0.747 +/- 0.059 g/cc. And, an ECM product consisting of toughened SIS laminate as described in U.S. Patent Application Serial No. XX/XXX,XXX entitled "Meniscus Regeneration Device and Method" (Attorney

10   Docket No. 265280-71141, DEP-745) has been made with a density of 0.933 +/- 0.061 g/cc.

         As discussed above, the ECM foams of the present disclosure may be combined with biological agents, bioactive agents, biologically derived substances, cells and/or stimulants, biocompatible inorganic materials and/or biocompatible

15   polymers (e.g., biocompatible synthetic polymers and biopolymers) and combinations of two or more of these materials at the time of manufacture. Illustratively, cells can be seeded throughout the three-dimensional volume of the ECM foam; the biological materials can be dried on the ECM foam at manufacture; the biological materials and the ECM foam can be co-lyophilized; and the biological materials can be covalently

20   linked to the ECM foam. It is contemplated to bond, cross-link, or otherwise incorporate one or more of these materials to the raw ECM material prior to formation of the ECM foam. Alternatively, the materials could be bonded, cross-linked, or otherwise incorporated to the final ECM foam after lyophilization. Finally, combinations of the above methods may be used. For example, an implant of

25   covalently linked ECM foam and a biological lubricant can be implanted and additional intra-articular injections of the same or different biological lubricants can be made at surgery, post-operatively, or both at surgery and post-operatively.

         In an illustrative embodiment, the biological agents are sequestered within the ECM foam of the present disclosure by physical entrapment. In one

30   exemplary process, the biological agent may be protected during such entrapment by liposomar delivery.

         In one aspect of this illustrative embodiment, the foam is cross-linked in the presence of the biological agent. For example, the foam may be incubated in

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the presence of the biological agent at a desired concentration. The foam is then cross-linked either chemically or enzymatically. Various types of cross-linking agents are known in the art and can be used such as, but not limited to, ribose and other sugars, oxidative agents and dehydrothermal methods. One exemplary cross-

5   linking agent is a water-soluble carbodiimide such as 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC). Sulfo-N-hydroxysuccinimide may be added to the EDC cross-linking agent as described by Staros, J.V., *Biochemistry* 21, 3950-3955 (1982). In one exemplary process the foam may be cross-linked in an aqueous solution comprising EDC at a concentration of

10   between about 0.1 mM to about 100 mM, more particularly between about 2.5 mM to about 25 mM. Excess reactants and by-products are removed by washing with a saline solution.

Alternatively, the foam may be enzymatically cross-linked. For example the foam may be cross-linked by the use of transglutaminase.

15   Transglutaminase catalyzes the covalent cross-linking of glutamine and/or lysine amino acid residues in the ECM foam. In this exemplary process the foam is first incubated with the biological agent. The foam is then immersed in an aqueous solution containing calcium chloride and transglutaminase. The temperature is raised to 37 °C for a period of from about 1 to about 24 hours. Excess reactants and by-

20   products are removed by washing with a saline solution. It should be appreciated that other enzymes may also be used to enzymatically cross-link the ECM foam. For example, the lysyl oxidase family of enzymes may be utilized.

It will be appreciated that the cross-linking agent is chosen such that it is non-reactive with the biological agent. For example, carbodiimide reacts with

25   hydroxyl, amine, or carboxylate groups while transglutaminase reacts with glutamine and lysine amino acid residues. Therefore, if the desired biological agents have any of these reactive groups either an alternative cross-linking agent is used, or the biological agent is perfused into the foam after cross-linking. The cross-linked foam is incubated (either statically or dynamically) in a solution of the biological agent for

30   a set period of time to allow for perfusion of the biological agent into the cross-linked foam. It will be appreciated that the incubation time will depend on the concentration of the biological agent as well as the amount of cross-linking of the foam. The lower

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the concentration of the biological agent and/or the greater the amount of cross-linking, the longer the incubation time.

In another aspect of the disclosure, the biological agent is released in a controlled manner from the foam. The rate of release is the rate of diffusion of the biological agent from the foam. The rate of release will be dependent on the concentration of the biological agent trapped in the foam and/or the degree of cross-linking of the foam. For example, FIG. 11 compares the release of FITC-labeled bovine serum albumin (BSA) from cross-linked and non-cross-linked foam. After 30 minutes approximately 80-100% of the FITC-BSA was released into solution from the non-cross-linked foam. In comparison, after 2 hours, only about 20% of the FITC-BSA was released from the cross-linked foam.

In another illustrative embodiment, the biological agents are covalently attached to the ECM foam. The biological agents may be attached directly or conjugated with a spacer arm. The method of covalent attachment will depend on the reactive groups of the biological agent, the reactivity of the amino acid side-chains of the ECM foam, and/or the reactivity of the spacer arm. For example, if the biological agent comprises a reactive hydroxyl, amino, or carboxylate group, a water soluble carbodiimide may be used to covalently attach the biological agent to the foam. Alternatively, if the biological agent is a peptide, protein, cell, or any other biological agent comprising a glutamine or lysine amino acid residue, the biological agent may be covalently attached to the foam by transglutiminase.

The covalently attached biological agents may be released in a controlled manner from the foam by resorbtion or other biological degradation of the foam. Alternatively, the biological agents may be released in a controlled manner by cleavage of the spacer arm attaching the agent to the foam. The spacer arm may be a cleavable linker that is cleavable by, but not limited to, reduction (i.e., a disulfide bond) or hydrolysis. Illustratively, the spacer arm may be cleaved where it attaches to the biological agent.

It will be appreciated by the skilled artisan that biological agents of the present disclosure can be physically trapped or covalently attached to different forms of ECM other than foams. Other forms of ECM include laminated, comminuted, or gel constitutions. As used herein, the term "biological agents" is intended to include bioactive agents, biologically-derived agents, and/or cells.

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"Bioactive agents" include one or more of the following: chemotactic agents; therapeutic agents (e.g., antibiotics, steroidal and non-steroidal analgesics and anti-inflammatories, anti-rejection agents such as immunosuppressants and anti-cancer drugs); various proteins (e.g., short chain peptides, bone morphogenic proteins, glycoprotein and lipoprotein); cell attachment mediators; biologically active ligands; integrin binding sequence; ligands; various growth and/or differentiation agents (e.g., epidermal growth factor, IGF-I, IGF-II, TGF-ß I-III, growth and differentiation factors, vascular endothelial growth factors, fibroblast growth factors, platelet derived growth factors, insulin derived growth factor and transforming growth factors, parathyroid hormone, parathyroid hormone related peptide, bFGF; TGFß superfamily factors; BMP-2; BMP-4; BMP-6; BMP-12; sonic hedgehog; GDF5; GDF6; GDF8; PDGF); small molecules that affect the upregulation of specific growth factors; tenascin-C; hyaluronic acid; chondroitin sulfate; fibronectin; decorin; thromboelastin; thrombin-derived peptides; heparin-binding domains; heparin; heparan sulfate; DNA fragments and DNA plasmids . If other such substances have therapeutic value in the orthopaedic field, it is anticipated that at least some of these substances will have use in concepts of the present disclosure, and such substances should be included in the meaning of "bioactive agent" and "bioactive agents" unless expressly limited otherwise.

"Biologically derived agents" include one or more of the following: bone (autograft, allograft, and xenograft) and derivates of bone; cartilage (autograft, allograft and xenograft), including, for example, meniscal tissue, and derivatives; ligament (autograft, allograft and xenograft) and derivatives; derivatives of intestinal tissue (autograft, allograft and xenograft), including for example submucosa; derivatives of stomach tissue (autograft, allograft and xenograft), including for example submucosa; derivatives of bladder tissue (autograft, allograft and xenograft), including for example submucosa; derivatives of alimentary tissue (autograft, allograft and xenograft), including for example submucosa; derivatives of respiratory tissue (autograft, allograft and xenograft), including for example submucosa; derivatives of genital tissue (autograft, allograft and xenograft), including for example submucosa; derivatives of liver tissue (autograft, allograft and xenograft), including for example liver basement membrane; derivatives of skin tissue; platelet rich plasma (PRP), platelet poor plasma, bone marrow aspirate, demineralized bone matrix,

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insulin derived growth factor, whole blood, fibrin and blood clot. Purified ECM and other collagen sources are also intended to be included within "biologically derived agents." If other such substances have therapeutic value in the orthopaedic field, it is anticipated that at least some of these substances will have use in the concepts of the present disclosure, and such substances should be included in the meaning of "biologically-derived agent" and "biologically-derived agents" unless expressly limited otherwise.

"Biologically derived agents" also include bioremodelable collageneous tissue matrices. The expressions "bioremodelable collagenous tissue matrix" and "naturally occurring bioremodelable collageneous tissue matrix" include matrices derived from native tissue selected from the group consisting of skin, artery, vein, pericardium, heart valve, dura mater, ligament, bone, cartilage, bladder, liver, stomach, fascia and intestine, tendon, whatever the source. Although "naturally occurring bioremodelable collageneous tissue matrix" is intended to refer to matrix material that has been cleaned, processed, sterilized, and optionally cross-linked, it is not within the definition of a naturally occurring bioremodelable collageneous tissue matrix to purify the natural fibers and reform a matrix material from purified natural fibers. The term "bioremodelable collageneous tissue matrices" includes "extracellular matrices" within its definition.

"Cells" include one or more of the following: chondrocytes; fibrochondrocytes; osteocytes; osteoblasts; osteoclasts; synoviocytes; bone marrow cells; mesenchymal cells; stromal cells; stem cells; embryonic stem cells; precursor cells derived from adipose tissue; peripheral blood progenitor cells; stem cells isolated from adult tissue; genetically transformed cells; a combination of chondrocytes and other cells; a combination of osteocytes and other cells; a combination of synoviocytes and other cells; a combination of bone marrow cells and other cells; a combination of mesenchymal cells and other cells; a combination of stromal cells and other cells; a combination of stem cells and other cells; a combination of embryonic stem cells and other cells; a combination of precursor cells isolated from adult tissue and other cells; a combination of peripheral blood progenitor cells and other cells; a combination of stem cells isolated from adult tissue and other cells; and a combination of genetically transformed cells and other cells. If other cells are found to have therapeutic value in the orthopaedic field, it is anticipated that at least some of

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these cells will have use in the concepts of the present disclosure, and such cells should be included within the meaning of "cell" and "cells" unless expressly limited otherwise.

"Biological lubricants" include: hyaluronic acid and its salts, such as sodium hyaluronate; glycosaminoglycans such as dermatan sulfate, heparan sulfate, chondroiton sulfate and keratan sulfate; synovial fluid and components of synovial fluid, including as mucinous glycoproteins (e.g., lubricin), vitronectin, tribonectins, articular cartilage superficial zone proteins, surface-active phospholipids, lubricating glycoproteins I, II; and rooster comb hyaluronate. "Biological lubricant" is also intended to include commercial products such as ARTHREASETM high molecular weight sodium hyaluronate, available in Europe from DePuy International, Ltd. of Leeds, England, and manufactured by Bio-Technology General (Israel) Ltd., of Rehovot, Israel; SYNVISC® Hylan G-F 20, manufactured by Biomatrix, Inc., of Ridgefield, New Jersey and distributed by Wyeth-Ayerst Pharmaceuticals of Philadelphia, Pennsylvania; HYLAGAN® sodium hyaluronate, available from Sanofi-Synthelabo, Inc., of New York, New York, manufactured by FIDIA S.p.A., of Padua, Italy; and HEALON® sodium hyaluronate, available from Pharmacia Corporation of Peapack, New Jersey in concentrations of 1%, 1.4% and 2.3% (for opthalmologic uses). If other such substances have therapeutic value in the orthopaedic field, it is anticipated that at least some of these substances will have use in the concepts of the present disclosure, and such substances should be included in the meaning of "biological lubricant" and "biological lubricants" unless expressly limited otherwise.

"Biocompatible polymers" is intended to include both synthetic polymers and biopolymers (e.g., collagen). Examples of biocompatible polymers include: polyesters of [alpha]-hydroxycarboxylic acids, such as poly(L-lactide) (PLLA) and polyglycolide (PGA); poly-p-dioxanone (PDS); polycaprolactone (PCL); polyvinyl alchohol (PVA); polyethylene oxide (PEO); polymers disclosed in United States Pat. Nos. 6,333,029 and 6,355,699; and any other bioresorbable and biocompatible polymer, co-polymer or mixture of polymers or co-polymers that are utilized in the construction of prosthetic implants. In addition, as new biocompatible, bioresorbable materials are developed, it is expected that at least some of them will be useful materials from which the anchors may be made. It should be understood that

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the above materials are identified by way of example only, and the present invention is not limited to any particular material unless expressly called for in the claims.

"Biocompatible inorganic materials" include materials such as hydroxyapatite, all calcium phosphates, alpha-tricalcium phosphate, beta-tricalcium

5   phosphate, calcium carbonate, barium carbonate, calcium sulfate, barium sulfate, polymorphs of calcium phosphate, ceramic particles, and combinations of such materials. If other such substances have therapeutic value in the orthopaedic field, it is anticipated that at least some of these substances will have use in the concepts of the present disclosure, and such substances should be included in the meaning of

10   "biocompatible inorganic material" and "biocompatible inorganic materials" unless expressly limited otherwise.

It is expected that various combinations of bioactive agents, biologically derived agents, cells, biological lubricants, biocompatible inorganic materials, biocompatible polymers can be used with the scaffolds of the present

15   disclosure.

It is expected that standard sterilization techniques may be used with the products of the present disclosure.

Illustratively, in one example of embodiments that are to be seeded with living cells such as chondrocytes, a sterilized implant may be subsequently

20   seeded with living cells and packaged in an appropriate medium for the cell type used. For example, a cell culture medium comprising Dulbecco's Modified Eagles Medium (DMEM) can be used with standard additives such as non-essential aminoacids, glucose, ascorbic acid, sodium pyrovate, fungicides, antibiotics, etc., in concentrations deemed appropriate for cell type, shipping conditions, etc.

25   It is anticipated that the ECM foams of the present disclosure may be combined with the concepts disclosed in the following applications for U.S. patent, filed concurrently herewith, which are incorporated by reference herein in their entireties: Serial No. XX/XXX,XXX entitled "Meniscus Regeneration Device and Method" (Attorney Docket No. 265280-71141, DEP-745); Serial No. XX/XXX,XXX

30   entitled "Devices from Naturally Occurring Biologically Derived Materials" (Attorney Docket No. 265280-71142, DEP-748); Serial No. XX/XXX,XXX entitled "Cartilage Repair Apparatus and Method" (Attorney Docket No. 265280-71143, DEP-749); Serial No. XX/XXX,XXX entitled "Unitary Surgical Device and Method"

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(Attorney Docket No. DEP-750); Serial No. XX/XXX,XXX entitled "Hybrid Biologic/Synthetic Porous Extracellular Matrix Scaffolds" (Attorney Docket No. 265280-71144, DEP-751); Serial No. XX/XXX,XXX entitled "Cartilage Repair and Regeneration Device and Method" (Attorney Docket No. 265280-71145, DEP-752);

5   Serial No. XX/XXX,XXX entitled "Porous Extracellular Matrix Scaffold and Method" (Attorney Docket No. 265280-71146, DEP-747); and Serial No. XX/XXX,XXX entitled "Cartilage Repair and Regeneration Scaffolds and Method" (Attorney Docket No. 265280-71180, DEP-763), along with U.S. Patent Application Serial No. 10/172,347 entitled "Hybrid Biologic-Synthetic Bioabsorbable Scaffolds"

10   which was filed on June 14, 2002. For example, for orthopaedic uses, it may be desirable to accompany or follow implantation with a treatment regime involving administering hyaluronic acid to the implantation site.

As can be seen from the forgoing description, the concepts of the present disclosure provide numerous advantages. For example, the concepts of the

15   present disclosure provide for the fabrication of a porous implantable scaffold which may have varying mechanical properties to fit the needs of a given scaffold design. For instance, the pore size and the material density may be varied to produce a scaffold having a desired mechanical configuration. In particular, such variation of the pore size and the material density of the scaffold is particularly useful when

20   designing a scaffold which provides for a desired amount of cellular migration therethrough, while also providing a desired amount of structural rigidity. In addition, according to the concepts of the present disclosure, implantable devices can be produced that not only have the appropriate physical microstructure to enable desired cellular activity upon implantation, but also has the biochemistry (collagens, growth

25   factors, glycosaminoglycans, etc.) naturally found in such ECMs.

Although it is believed that naturally occurring extracellular matrix provides advantages over purified extracellular matrix, it is contemplated that the teachings of the present disclosure can be applied to purified extracellular matrix as well. Thus, it is expected that the naturally occurring extracellular matrix could be

30   purified prior to physically comminuting the extracellular matrix. This purification could comprise treating the naturally occurring extracellular matrix to remove substantially all materials other than collagen prior to physically comminuting the extracellular matrix. The purification could be carried out to substantially remove

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glycoproteins, glycosaminoglycans, proteoglycans, lipids, non-collagenous proteins and nucleic acids (DNA and RNA).

While the disclosure is susceptible to various modifications and alternative forms, specific exemplary embodiments thereof have been shown by way

5   of example in the drawings and has herein be described in detail. It should be understood, however, that there is no intent to limit the disclosure to the particular forms disclosed, but on the contrary, the intention is to cover all modifications, equivalents, and alternatives falling within the spirit and scope of the disclosure.

There are a plurality of advantages of the present disclosure arising

10   from the various features of the apparatus and methods described herein. It will be noted that alternative embodiments of the apparatus and methods of the present disclosure may not include all of the features described yet still benefit from at least some of the advantages of such features. Those of ordinary skill in the art may readily devise their own implementations of an apparatus and method that incorporate

15   one or more of the features of the present disclosure and fall within the spirit and scope of the present disclosure.

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CLAIMS:

1.   A method of making an implantable scaffold for repairing or regenerating body tissue, the method comprising the steps of:

        suspending pieces of a naturally occurring extracellular matrix material in a liquid;

        freeze drying the pieces of naturally occurring extracellular matrix material and the liquid; and

        incorporating at least one biological agent into the freeze dried extracellular matrix material.

2.   The method of claim 1, further comprising the step of freezing the extracellular matrix material and the liquid to form ice crystals from the liquid, the freezing step being performed prior to the freeze drying step.

3.   The method of claim 2, wherein the freeze drying step further comprises subliming the ice crystals directly to vapor in the presence of a vacuum.

4.   The method of claim 1, wherein the freeze drying step comprises subliming the liquid so as to form a porous body.

5.   The method of claim 1, further comprising the step of comminuting the extracellular matrix material into the pieces.

6.   The method of claim 1, wherein the extracellular matrix material comprises material selected from the group consisting of: small intestine submucosa, bladder submucosa, stomach submucosa, alimentary submucosa, respiratory submucosa, genital submucosa, and liver basement membrane

7.   The method of claim 1, further comprising the step of flash-freezing the extracellular matrix material and the liquid prior to the freeze drying step.

8.   The method of claim 1, further comprising the step of compacting the pieces of naturally occurring extracellular matrix material prior to the freeze drying step.

9.   The method of claim 1, further comprising the step of centrifuging the pieces of naturally occurring extracellular matrix material prior to the freeze drying step.

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10.   The method of claim 1, further comprising the step of freezing the naturally occurring extracellular matrix material and the liquid at a controlled rate of temperature drop.

11.   The method of claim 10, wherein the freezing step comprises varying the rate of temperature drop so as to vary the pore size of the scaffold.

12.   The method of claim 1, wherein the incorporating step comprises:

incubating the freeze dried extracellular matrix material with a solution comprising at least one biological agent; and

cross-linking the freeze dried extracellular matrix material.

13.   The method of claim 12, wherein cross-linking the freeze dried extracellular matrix material comprises cross-linking the freeze dried extracellular matrix material by use of a water-soluble carbodiimide.

14.   The method of claim 12, wherein cross-linking the freeze dried extracellular matrix material comprises cross-linking the freeze dried extracellular matrix material by use of transglutaminase.

15.   The method of claim 1, wherein the incorporating step comprises covalently attaching the biological agent to the extracellular matrix material.

16.   The method of claim 1, wherein the biological agent is selected from the group consisting of a bioactive agent, a biologically-derived agent, and a cell.

17.   An implantable scaffold for repairing or regenerating body tissue, comprising:

a porous body which is prepared by a process comprising the steps of:

suspending pieces of a naturally occurring extracellular matrix material in a liquid,

freeze drying the naturally occurring extracellular matrix material and the liquid; and

incorporating at least one biological agent into the freeze dried extracellular matrix material.

18.   The implantable scaffold of claim 17, wherein the process for preparing the porous body further comprises the step of freezing the naturally

occurring extracellular matrix material and the liquid to form ice crystals from the liquid, the freezing step being performed prior to the freeze drying step.

19.   The implantable scaffold of claim 18, wherein the freeze drying step further comprises subliming the ice crystals directly to vapor in the presence of a vacuum.

20.   The implantable scaffold of claim 17, wherein the freeze drying step comprises subliming the liquid.

21.   The implantable scaffold of claim 17, wherein the process for preparing the porous body further comprises the step of comminuting the naturally occurring extracellular matrix material into the pieces.

22.   The implantable scaffold of claim 17, wherein the naturally occurring extracellular matrix material comprises material selected from the group consisting of: small intestine submucosa, stomach submucosa, bladder submucosa, alimentary submucosa, respiratory submucosa, genital submucosa, and liver basement membrane.

23.   The implantable scaffold of claim 17, wherein the process for preparing the porous body further comprises the step of flash-freezing the naturally occurring extracellular matrix material and the liquid prior to the freeze drying step.

24.   The implantable scaffold of claim 17, wherein the process for preparing the porous body further comprises the step of compacting the pieces of naturally occurring extracellular matrix material prior to the freeze drying step.

25.   The implantable scaffold of claim 17, wherein the process for preparing the porous body further comprises the step of centrifuging the pieces of naturally occurring extracellular matrix material prior to the freeze drying step.

26.   The implantable scaffold of claim 17, wherein the incorporating step comprises:

incubating the freeze dried extracellular matrix material with a solution comprising at least one biological agent; and

cross-linking the freeze dried extracellular matrix material.

27.   The implantable scaffold of claim 26, wherein cross-linking the freeze dried extracellular matrix material comprises cross-linking the freeze dried extracellular matrix material by use of a water-soluble carbodiimide.

28.   The implantable scaffold of claim 26, wherein cross-linking the freeze dried extracellular matrix material comprises cross-linking the freeze dried extracellular matrix material by use of transglutaminase.

29.   The implantable scaffold of claim 17, wherein the incorporating step comprises covalently attaching the biological agent to the extracellular matrix material.

30.   The implantable scaffold of claim 17, wherein the biological agent is selected from the group consisting of a bioactive agent, a biologically-derived agent, and a cell.

31.   A method of making an implantable device for repairing or regenerating body tissue, the method comprising the steps of:

    providing a naturally occurring extracellular matrix material in a raw form;

    comminuting the raw naturally occurring extracellular matrix in the presence of a liquid to form a slurry of naturally occurring extracellular matrix;

    lyophilizing the slurry of naturally occurring extracellular matrix to form an open cell foam of naturally occurring extracellular matrix; and

    incorporating at least one biological agent into the open cell foam.

32.   The method of claim 31, wherein the open cell foam of naturally occurring extracellular matrix includes molecules other than collagen, said molecules other than collagen being present in the raw form of the naturally occurring extracellular matrix.

33.   The method of claim 31, wherein the incorporating step comprises:

    incubating the freeze dried extracellular matrix material with a solution comprising at least one biological agent; and

    cross-linking the freeze dried extracellular matrix material.

34.   The method of claim 33, wherein cross linking the freeze dried extracellular matrix material comprises cross-linking the freeze dried extracellular matrix material by use of a water-soluble carbodiimide.

35.   The method of claim 33, wherein cross linking the freeze dried extracellular matrix material comprises cross-linking the freeze dried extracellular matrix material by use of transglutaminase.

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36. The method of claim 31, wherein the incorporating step comprises covalently attaching the biological agent to the extracellular matrix material.

37. The method of claim 31, wherein the biological agent is selected from the group consisting of a bioactive agent, a biologically-derived agent, and a cell.

38. A extracellular matrix foam comprising a biological agent.

39. The foam of claim 38, wherein the biological agent is selected from the group consisting of a bioactive agent, a biologically-derived agent, and a cell.

40. A method of making an extracellular matrix foam comprising the step of incorporating a biological agent into the foam.

41. The method of claim 40, wherein the incorporating step comprises:

        incubating the foam with a solution comprising at least one biological agent; and

        cross-linking the foam.

42. The method of claim 41, wherein cross linking the foam comprises cross-linking the foam by use of a water-soluble carbodiimide.

43. The method of claim 41, wherein cross linking the foam comprises cross-linking the foam by use of transglutaminase.

44. The method of claim 40, wherein the incorporating step comprises covalently attaching the biological agent to the foam.

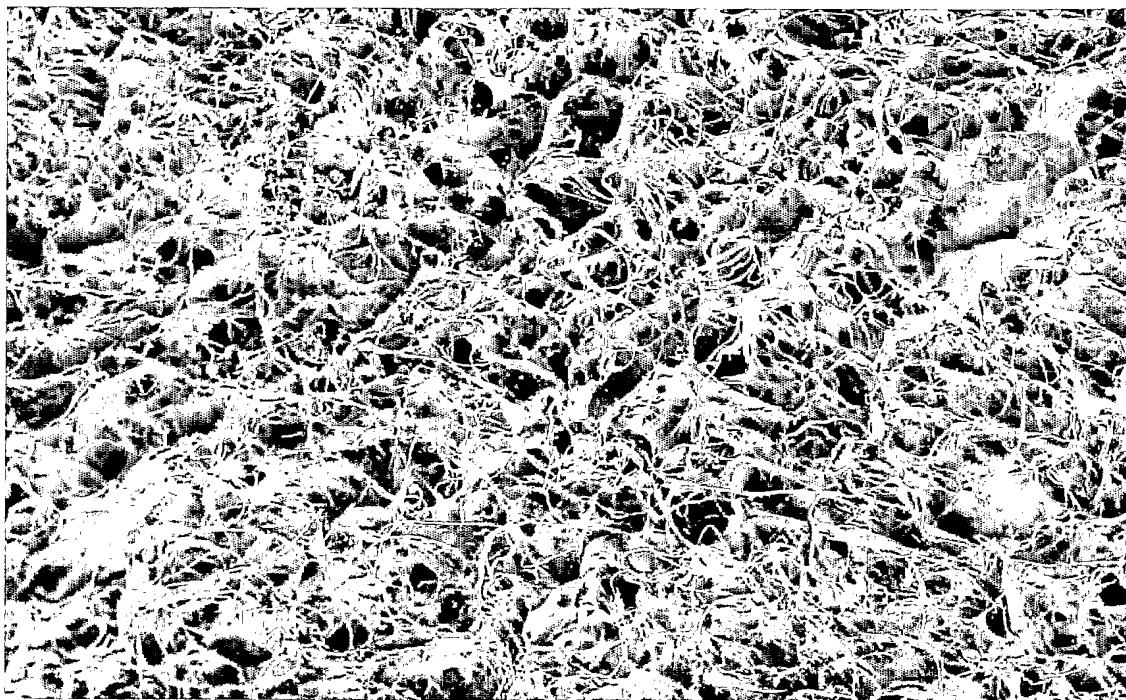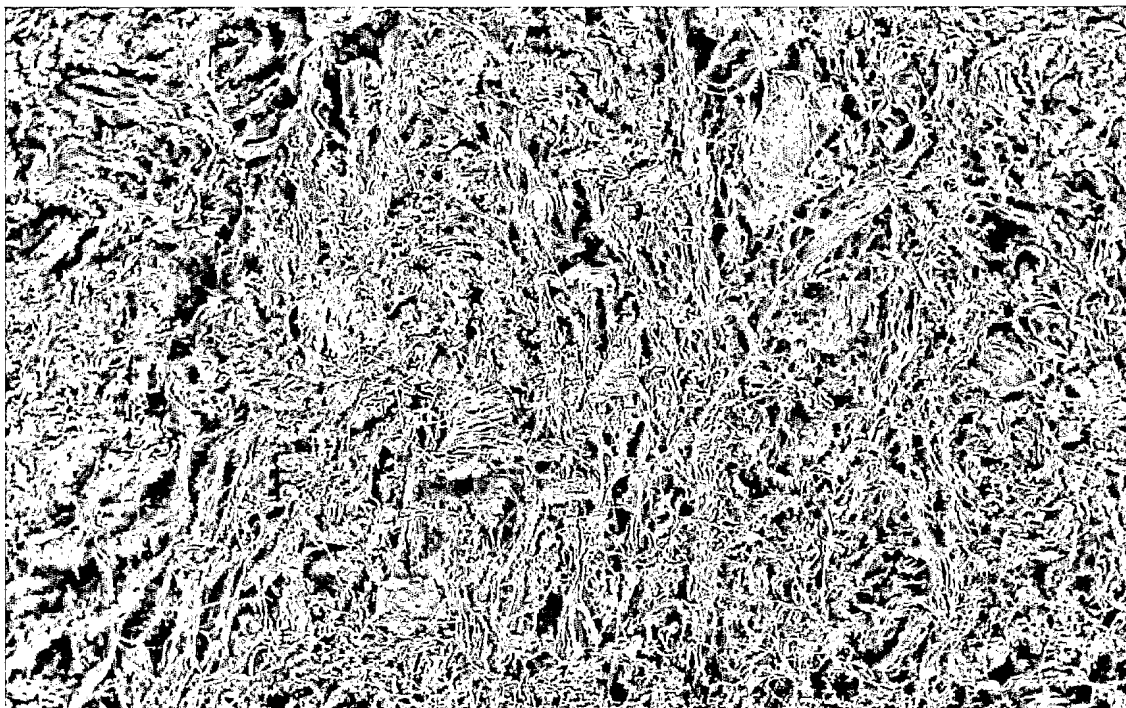45. The method of claim 40, wherein the biological agent is selected from the group consisting of a bioactive agent, a biologically-derived agent, and a cell.
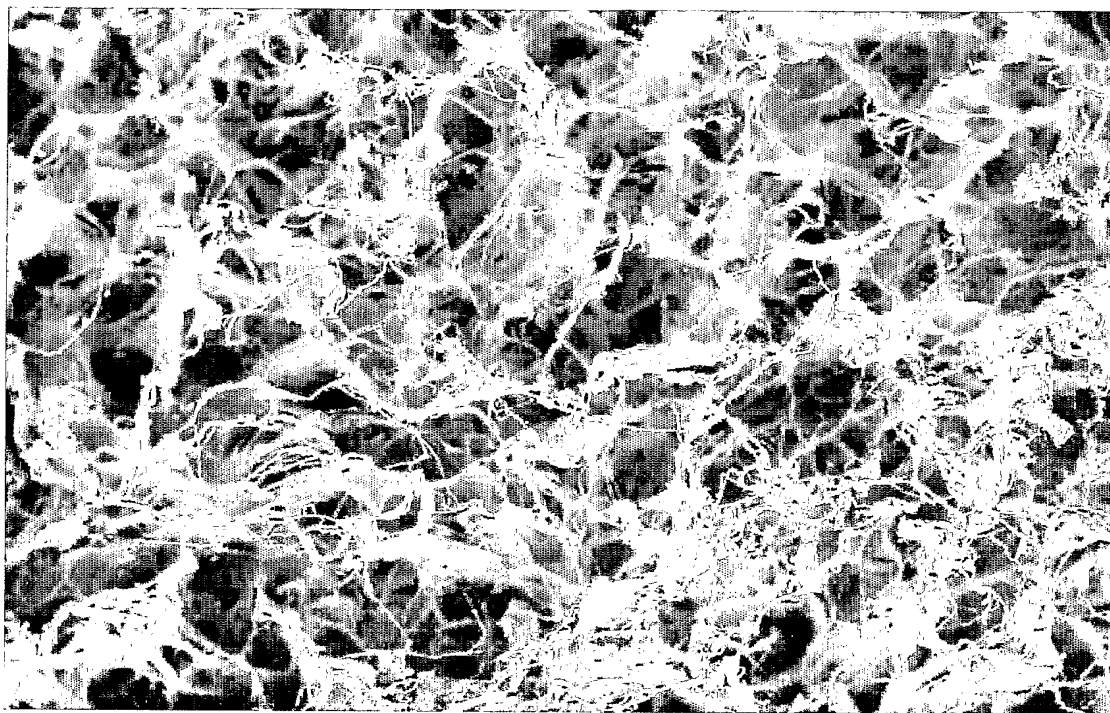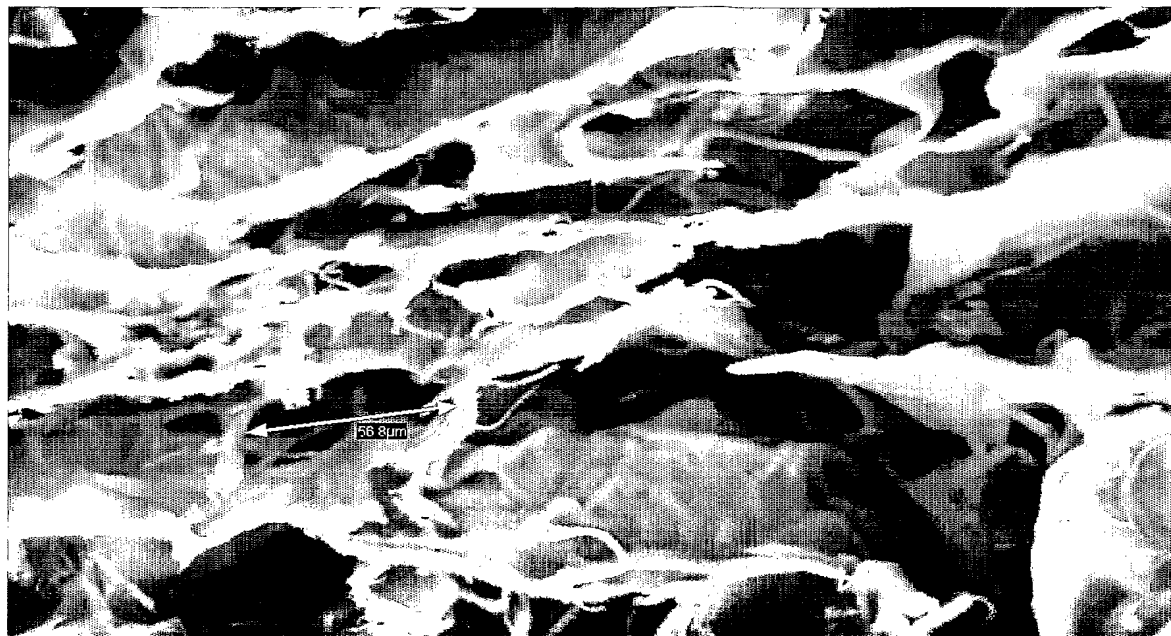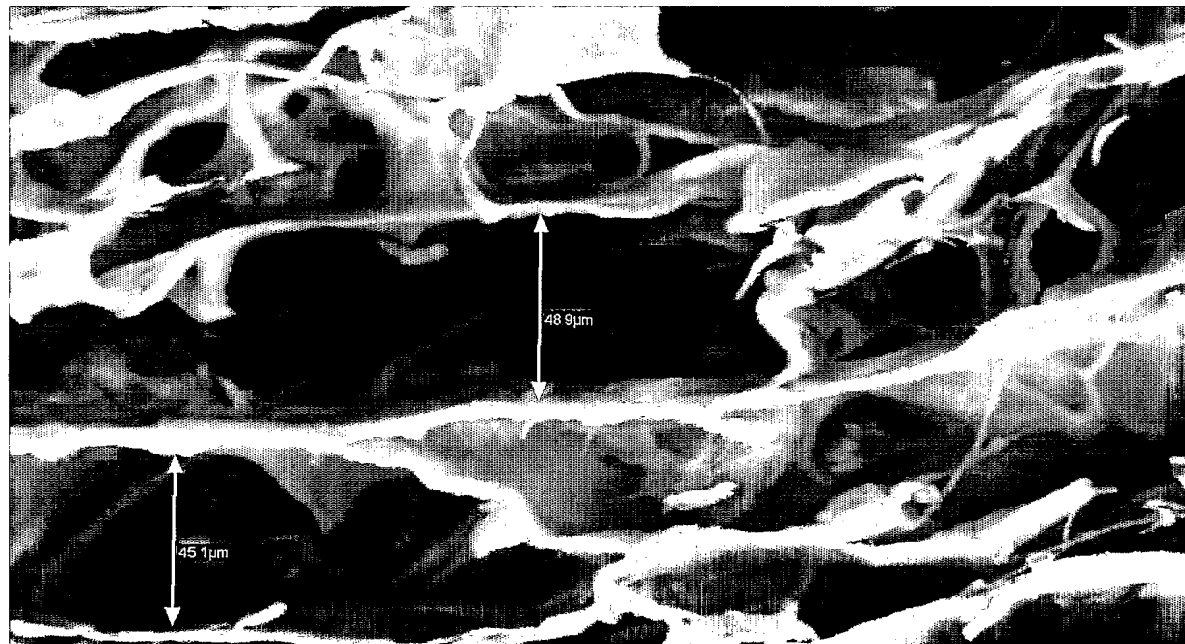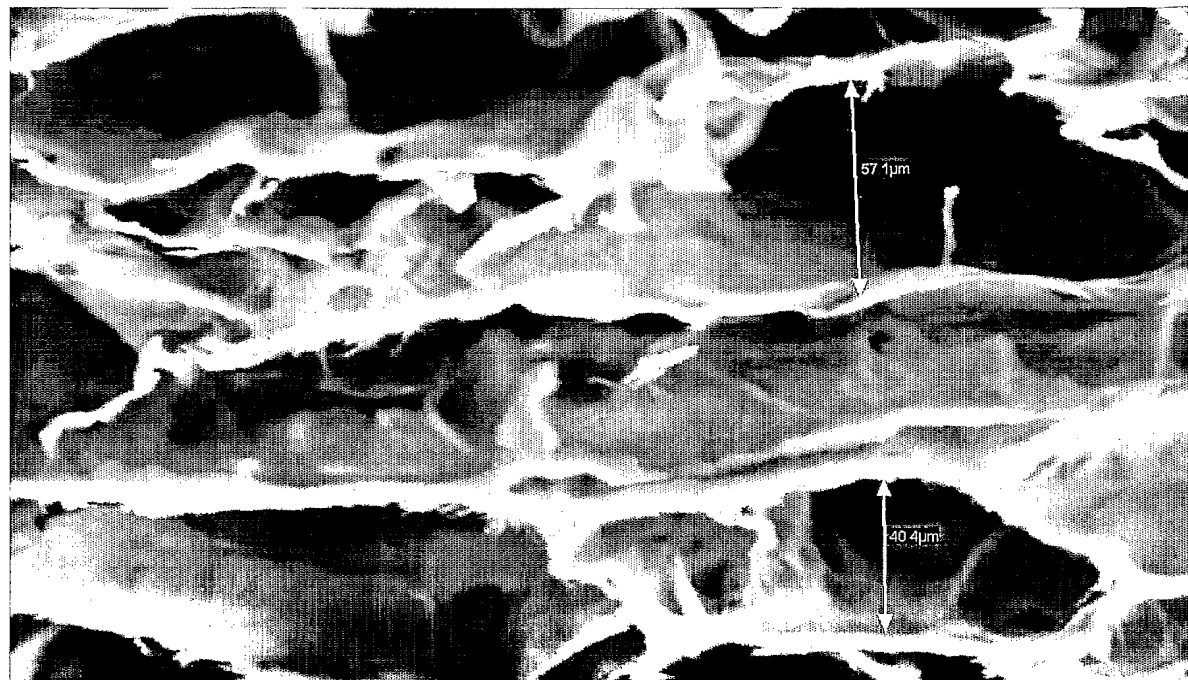
Fig. 1
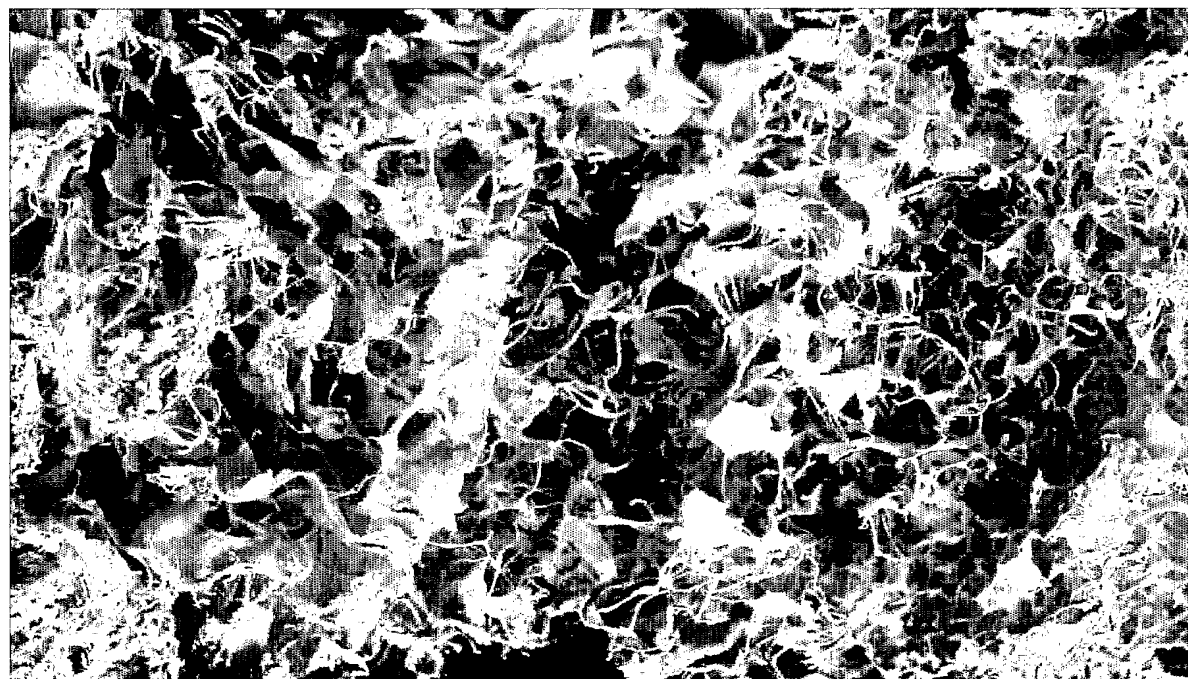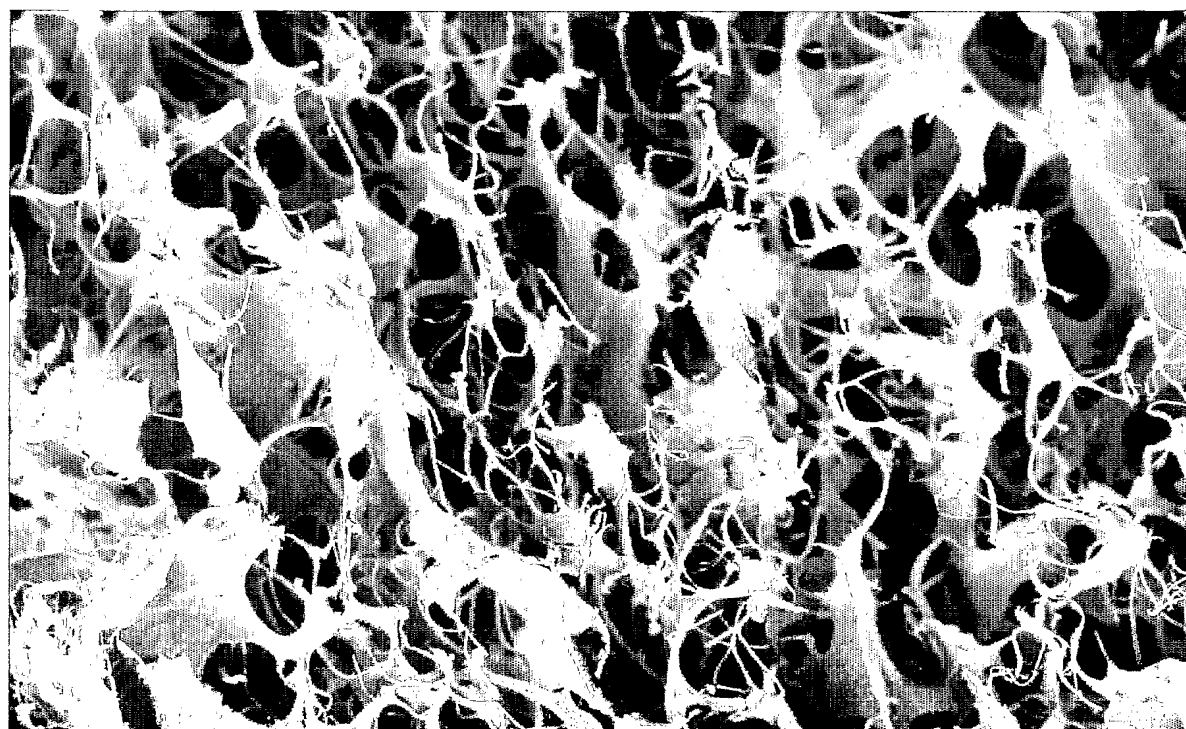
Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

Fig. 8
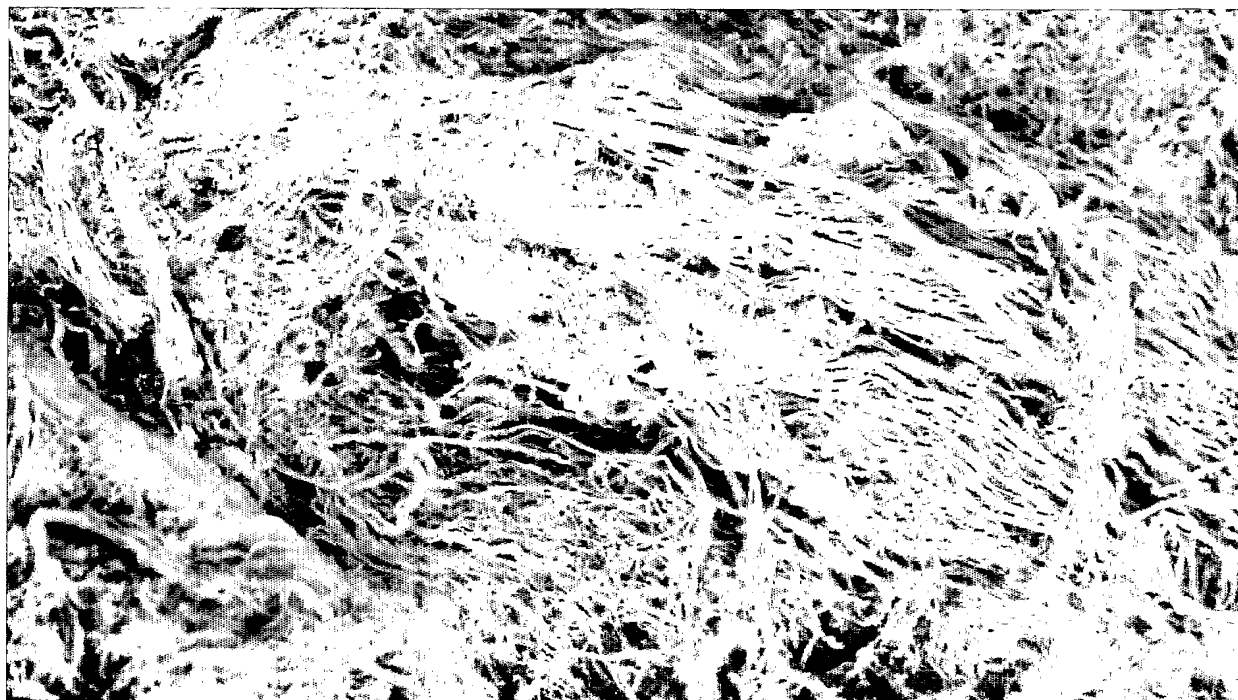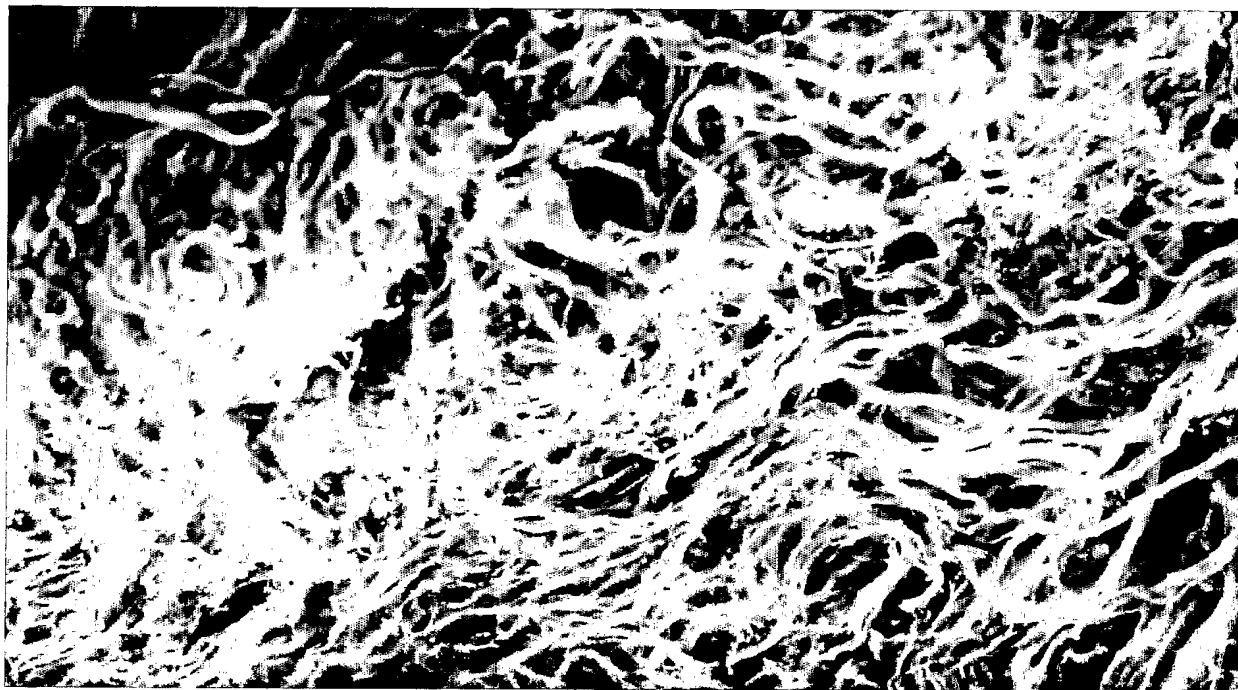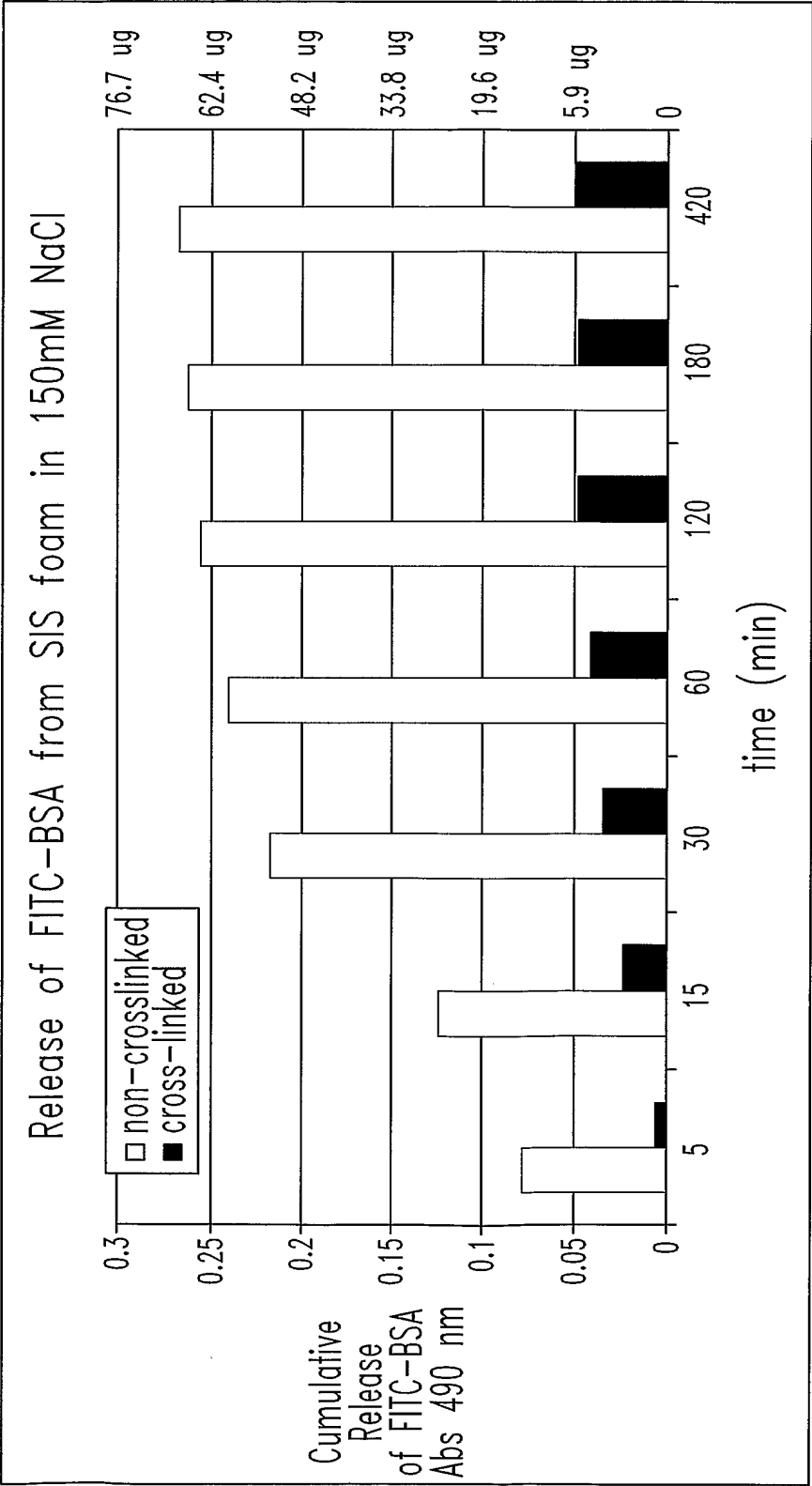
Fig. 9

Fig. 10

Cumulative Release of FITC–BSA Over Time

Release of FITC–BSA from SIS foam in 150mM NaCl

Legend:
□ non-crosslinked
■ cross-linked

y-axis: Cumulative Release of FITC–BSA Abs 490 nm — 0, 0.05, 0.1, 0.15, 0.2, 0.25, 0.3

Right labels: 0, 5.9 ug, 19.6 ug, 33.8 ug, 48.2 ug, 62.4 ug, 76.7 ug

x-axis: time (min) — 5, 15, 30, 60, 120, 180, 420

Fig. 11